

Diagram of the pCMV-gp140.BX08 plasmid construct. The plasmid is circular, 2.6 kb in size. It features a CMV promoter (1.6 kb) and a gp140 gene (2.0 kb). The gp140 gene is flanked by TPA, NheI, and BamHI sites. The plasmid also contains a bGHpA gene (0.2 kb) flanked by PstI and XhoI sites, and a KanR resistance gene. The plasmid is labeled pCMV-gp140.BX08.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

TITLE OF INVENTIONEXPRESSING GP140 FRAGMENT OF PRIMARY HIV-1 ISOLATEFIELD OF INVENTION

The present invention relates to the field of immunology, specifically HIV  
5 Vaccine Technology, and, in particular, is concerned with expressing the  
extracellular fragment of the envelope gene, gp140, of a primary human  
immunodeficiency virus type 1 (HIV-1) isolate.

REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of copending United States  
10 Patent Application No.: 09/256,194 filed February 24, 1999.

BACKGROUND OF THE INVENTION

Acquired immunodeficiency syndrome (AIDS) is a disease which is the  
ultimate result of infection with human immunodeficiency virus (HIV). Currently,  
there is no effective vaccine which can protect the human population from HIV  
15 infection and hence the development of an efficacious HIV-vaccine and protocol  
for administering the same is urgently required. Previously, HIV-1 particles  
exhaustively inactivated by chemical treatments, a vaccinia vector encoding the  
whole envelope gene (gp140) of HIV-1, and purified recombinant gp120 have  
been evaluated as candidate HIV vaccines. Although inactivated HIV-1 virus  
20 preparations elicited a T-cell-mediated Delayed-Type Hypersensitivity (DTH)  
reaction in humans, and vaccinia/gp160 and gp120 recombinant vaccine  
candidates induced virus neutralizing antibodies, none of these immunogens have  
been shown to be efficacious human HIV vaccines (ref. 1, throughout this  
specification, various references are referred to in parenthesis to more fully describe  
25 the state of the art to which this invention pertains. Full bibliographic information  
for each citation is found at the end of the specification, immediately following the  
claims. The disclosures of these references are hereby incorporated by reference  
into the present disclosure). The inventors' interest in HIV vaccinology is to  
develop immunogenic and cost-effective HIV-1 DNA vaccines and consider that

their use alone or in conjunction with other forms of HIV-1 vaccine candidates will lead to the elicitation of more effective immune responses against HIV-1.

There has previously been described in granted European Patent No. 470,980 and U.S. Patent No. 5,639,854, assigned to the assignee hereof, the disclosures of which are incorporated herein by reference, *inter alia*, the identification and characterization of a T-cell epitope of the core protein, p24E, of HIV-1. There has further been described in granted U.S. Patents Nos. 5,759,769 and 5,795,955, assigned to the assignee hereof, and disclosures of which are incorporated by reference, the use of the T-cell epitope in the construction of immunogenic synthetic HIV-1 chimeric peptides comprising p24E linked to amino acid sequences of different B-cell epitopes of an envelope or core protein of HIV-1.

#### SUMMARY OF THE INVENTION

The present effort has turned to design and construction of HIV DNA-based immunogens capable of eliciting cell-mediated immunity (CMI). In this context, the inventors have focused interest on the extracellular envelope fragment, gp140, expressed in a primary HIV-1 isolate, HIV-1 (BX08), for the reason that this protein is rich in motifs restricted to both the murine and human Major Histocompatibility Complex (MHC) class 1 alleles.

Immunization with an appropriately constructed immunogen expressing the gp140 protein leads to the generation of peptides with class 1 binding capability to allow the induction of HIV-1-specific CTLs capable of killing virus infected cells to limit infection.

The invention described by the inventors is that they have found a plasmid designated, pCMV.gp140.BX08, expressing the gp140 gene under the control of a CMV promotor was immunogenic in BALB/c mice in the elicitation of CTL response directed against multiple epitopes of the gp140 protein that are restricted to different H-2<sup>d</sup> class 1 gene products. It was also found that plasmids based on Semliki Forest Virus (SFV) vectors, namely pMP83, pMP84 and pMP88, also requiring the gp140 gene under the control of a CMV promoter were similarly immunogenic.

Accordingly, in one aspect of the present invention, there is provided a vector, comprising a gene encoding the extracellular fragments of gp140 of a primary HIV-1 isolate, preferably BX08, under the control of a promotor for expression of the gene product in a host organism, thereby eliciting a cytotoxic T-cell response.

The promotor preferably is the cytomegalovirus promotor. The vector may preferably be a plasmid vector having the identifying characteristics of plasmid pCMV.gp140.BX08, as shown in Figure 1. The vector also may preferably be a plasmid vector having the identifying characteristics of plasmids pMP88, pMP84 or pMP83.

The invention further includes an immunogenic composition containing the vector as well as a method of generating a cytotoxic T-cell response to HIV-1 in a host by administering to the host the immunogenic composition provided herein. Such immunogenic composition may be formulated for intramuscular immunization with a suitable carrier or may be formulated for gene gun delivery with gold particles.

The invention extends to the vector when used as an immunogen for generating a cytotoxic T-cell response to HIV-1 in a host and to the use of the vector in the manufacture of an immunogen for the generation of a cytotoxic T-cell response to the HIV-1 in a host.

The invention further includes certain novel peptides and nucleic acid molecules as set forth below.

#### BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows details of the elements of plasmid pCMV.gp140.BX08;

Figure 2 shows the nucleotide (SEQ ID No: 1) and deduced amino acid sequence (SEQ ID No: 2) of the gp140 open reading frame of the plasmid pCMV.gp140.BX08;

Figures 3A, 3B and 3C show the effector responses elicited by intramuscular injection of the plasmid, pCMV.gp140.BX08, in BALB/c mice;

Figures 4A, 4B and 4C show the effector responses elicited by gene gun delivery of the plasmid, pCMV.gp140.BX08, in BALB/c mice; and

Figure 5 is a nucleotide sequence of a BamHI fragment (SEQ ID No: 15) containing the HIV-1, gp140 nucleotide sequence for incorporation into a BamHI site of alphavirus expression vectors.

#### DETAILED DESCRIPTION OF THE INVENTION

5 A DNA immunogen is constructed using recombinant DNA technology to molecularly clone a gene of interest into a plasmid expression vector. A unique feature involving vaccination with a DNA-based immunogen is that, once delivered into a cell, the intracellular production of the immunogen favours the induction of MHC class 1-restricted cytotoxic T-cells as compared to other forms  
10 of vaccination involving the use of killed whole cell and formulated sub-unit immunogens, which tend to favour the elicitation of MHC class 2-restricted immuno-regulatory responses in the majority of cases studied (ref. 2). In this context, it is, therefore, favourable to use DNA technology to construct naked DNA immunogens for vaccination purposes in order to optimize the induction of  
15 cellular effector response against intracellular organisms, such as viruses as well as certain tumours. The other advantages DNA vaccines offer include: (i) the ease to produce them; and (ii) their stability over a wide temperature range.

A common model which has been used recently to predict murine and human CTL antigenic determinants has involved the identification of binding  
20 motifs for the respective MHC class 1 molecules from the primary sequences of the native protein molecules (see refs. 3 to 5). Thus, it has been proposed that motifs which are most favoured to bind and lodge into the peptide-binding groove of the H-2D<sup>d</sup> gene product is usually 8 to 10 amino acids long. In the majority of cases, these peptides are found to contain anchor residues, such as glycine and  
25 proline (GP), at positions 2 and 3 near the amino- (N-) terminus, and either a leucine or phenylalanine at the carboxy- (C-) terminus, which serve to interact with the respective 'pockets' of the peptide-binding groove of a membrane-bound H-2D<sup>d</sup> molecule. The motifs restricted to the other class 1 allele, K<sup>d</sup>, of the H-2<sup>d</sup> haplotype were reported to contain a tyrosine at position 2, and could be an  
30 isoleucine, valine or leucine at the C-terminus. Studies of the peptides isolated from the human MHC class 1 molecules, HLA-A0201, had similarly revealed that

the anchor residues were leucine or methionine at position 2 and valine or leucine at the C-terminus in the majority of cases.

The suitability of the HIV-1(BX08) gp140 gene product as a CTL-inducing immunogen was assessed by prediction algorithms to determine the number of both the murine and human MHC class 1-restricted binding motifs it contained. The amino acid sequences of the binding motifs and the designation of the peptides representing them are shown in Table 1 below. Such peptides are novel and are claimed herein. The presence of binding motifs towards the different H-2<sup>d</sup> restricted class 1 alleles, i.e. D<sup>d</sup> and K<sup>d</sup>, allows the immunogenicity of plasmids, pCMV.gp140.BX08, pMP83, pMP84 and pMP88, expressing gp140 of HIV-1 of the primary isolate, BX08, and constructed as described in the Examples below, to be studied in the inbred mouse strain BALB/c of the H-2<sup>d</sup> haplotype. The elements and restriction sites of plasmid pCMV.gp140.BX08 are shown in Figure 1. The construction of the plasmids pMP83, pMP84 and pMP88 is described in Example 5 below. The nucleotide sequence (SEQ ID No: 1) and the deduced amino acid sequence (SEQ ID No: 2) of the gp140 open reading frame of the plasmid pCMV.gp140.BX08, pMP83, pMP84 and pMP88, is shown in Figure 2, which appear to be unique sequences and are claimed herein along with their complements.

The location of several binding motifs against the human MHC class 1 allele, HLA-A0201, as seen in Table 1, implied that, under an appropriate immunization regimen, the plasmid has the potential to elicit CTL response directed to these epitopes in the context of this class 1 molecule in human subjects.

The immunogenicity of the plasmid pCMV.gp140.BX08 was studied in BALB/c mice. The results of the study involving three injections of the plasmid at 100.0 µg per dose using the intramuscular route are shown in Figure 3. Upon *in vitro* re-stimulation of the spleenocytes of the plasmid-immunized animals with irradiated autologous LPS blasts pulsed individually with the D<sup>d</sup>- and K<sup>d</sup>-restricted motif containing peptides, namely, CLP-501 and CLP-504 (SEQ ID Nos.: 3, 5), respectively, it was found that CTLs were generated that killed P815 targets presented with the respective peptides (Figs. 3A and 3B). The amino acid

sequences of the peptides are shown in Table 1. A comparison of the magnitude of the responses at the same effector to target (E:T) ratio revealed that the D<sup>d</sup>-restricted response to the CLP-501 peptide is immuno-dominant and that the K<sup>d</sup>-restricted response to the CLP-504 peptide is sub-dominant. The *in vitro* re-stimulation leading to the expansion of the effectors was specific because the addition of the same number of irradiated LPS blasts alone (not treated with peptide) did not lead to any generation of effectors in the bulk culture able to kill either of the specific targets tested. The findings that the control group of mice injected with the pCMV vector without the gp140 insert alone failed to generate any of the two sub-populations of CTLs (Fig. 3C) confirmed that the plasmid, pCMV.gp140.BX08, was indeed immunogenic.

The pCMV.gp140.BX08 plasmid, when delivered with the gene gun, was similarly found to be immunogenic. The results shown in Figure 4 show that following two injections at a dose of 0.7 µg of the plasmid, and using the same *in vitro* re-stimulation condition described above that CTLs recognizing the CLP-501 and CLP-504 peptides were detected (Figures 4A and 4B), while no effector response was elicited by the group of animals given the vector, pCMV, alone (Figure 4C).

The immunogenicity of the plasmids pMP83, pMP84 and pMP88 was separately studied, delivered intramuscularly, in BALB/c mice, in comparison to pCMV.gp140.BX08, at three different dosage levels, namely 1.0, 10.0 and 100.0 µg of DNA following the above described procedures. The results obtained are contained in Tables II and III below. CTL activation is achieved at significantly lower doses of the alphavirus vectors than with pCMV.gp140.BX08.

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination, diagnosis and treatment of HIV infections. A further non-limiting discussion of such uses is further presented below.

Immunogenic compositions, including vaccines, containing the DNA vector may be prepared as injectables, in physiologically-acceptable liquid solutions or emulsions for polynucleotide administration. The DNA vector may be associated with liposomes, such as lecithin liposomes or other liposomes



known in the art, as a nucleic acid liposome (for example, as described in WO 93/24640) or the DNA vector may be associated with an adjuvant, as described in more detail below.

Liposomes comprising cationic lipids interact spontaneously and rapidly with polyanions, such as DNA and RNA, resulting in liposome/nucleic acid complexes that capture up to 100% of the polynucleotide. In addition, the polycationic complexes fuse with cell membranes, resulting in an intracellular delivery of polynucleotide that bypasses the degradative enzymes of the lysosomal compartment. Published PCT application WO 94/27435 describes compositions for genetic immunization comprising cationic lipids and polynucleotides.

Agents which assist in the cellular uptake of nucleic acid, such as calcium ions, viral proteins and other transfection facilitating agents, may advantageously be used with the vector.

Polynucleotide immunogenic preparations may also be formulated as microcapsules, including biodegradable time-release particles. Thus, U.S. Patent 5,151,264 describes a particular carrier of a phospholipid/glycolipid/polysaccharide nature that has been termed Bio Vecteurs Supra Moléculaires (BVSM). The particulate carriers are intended to transport a variety of molecules having biological activity in one of the layers thereof.

U.S. Patent 5,075,109 describes encapsulation of the antigens trinitrophenylated keyhole limpet hemocyanin and staphylococcal enterotoxin B in 50:50 poly (DL-lactide-co-glycolide). Other polymers for encapsulation are suggested, such as poly(glycolide), poly(DL-lactide-co-glycolide), copolyoxalates, polycaprolactone, poly(lactide-co-caprolactone), poly(esteramides), polyorthoesters and poly(8-hydroxybutyric acid), and polyanhydrides.

Published PCT application WO 91/06282 describes a delivery vehicle comprising a plurality of bioadhesive microspheres and antigens. The microspheres being of starch, gelatin, dextran, collagen or albumin. This delivery vehicle is particularly intended for the uptake of vaccine across the nasal mucosa. The delivery vehicle may additionally contain and absorption enhancer.

In particular embodiments of the present invention, the vector may be delivered in conjunction with a targeting molecule to target the vector to selected cells including cells of the immune system.

The vectors may be delivered to the host by a variety of procedures, for example, Tang et al (ref. 6) discloses that introduction of gold microprojectiles coated with DNA encoding bovine growth hormone (BGH) into the skin of mice resulted in production of anti-BGH antibodies in the mice, while Furth et al. (ref. 7) showed that a jet injector could be used to transfect skin, muscle, fat and mammary tissues of living animals.

#### Biological Deposits

Certain vectors that contain nucleic acid coding for an extracellular fragment of gp140 of a primary isolate that are described and referred to herein as well as precursor alphavirus vectors have been deposited with the America Type Culture Collection (ATCC) located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA, pursuant the Budapest Treaty and prior to the filing of this application. Samples of the deposited vectors will become available to the public and all restrictions imposed or access to the deposits will be received upon grant of a patent based on this United States patent application. In addition, the deposit will be replaced if viable samples cannot be dispensed by the Depository. The invention described and claimed herein is not limited in scope by the biological materials deposited, since the deposited embodiment is intended only as an illustration of the invention. Any equivalent or similar vectors that contain nucleic acid which encodes equivalent or similar antigens as described in this application are within the scope of the invention.

#### Deposit Summary

Plasmid	ATCC	Deposited Date
pCMV.gp140.BX08	203839	March 9, 1999
25 pMP42	203461	November 12, 1998
pMP76	203462	November 12, 1998

#### EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific

Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms  
5 are intended in a descriptive sense and not for purposes of limitation.

Methods of plasmid construction, peptide synthesis, cell culture, CTL assay and other testing procedures that are not explicitly described in this disclosure are amply reported in the scientific literature and are well within the scope of those skilled in the art.

10 Example 1

This Example illustrates the construction of the plasmid, pCMV.gp140.BX08.

The plasmid, pCMV.gp140.BX08, contains sequence segments from various sources, and the elements of construction are depicted in Figure 1. The  
15 nucleotide (SEQ ID No: 1) and derived amino acid sequences (SEQ ID No: 2) of the gp140 open reading frame of the plasmid are shown in Figure 2.

The prokaryotic vector pBluescript SK (Stratagene) is the backbone of the plasmid pCMV.gp140.BX08 and was modified by the replacement of the Amp<sup>R</sup> with Kan<sup>R</sup> gene and the deletion of the fl and the LacZ regions. To achieve the  
20 desired modifications, the sequence between AhdI (nucleotide 2,041) and SacI (nucleotide 759) of pBluescript SK, which contains the Amp<sup>R</sup>, fl origin and the LacZ, was deleted. A 1.2 kb PstI fragment from the plasmid pUC-4K (Pharmacia) containing the Kan<sup>R</sup> gene, was blunt end ligated to the AhdI site of pBluescript SK in a counter-clockwise orientation relative to it's transcription. A 1.6 kb SspI/PstI  
25 DNA fragment containing the human cytomegalovirus immediate-early gene promotor, enhancer and intron A sequences (CMV) was ligated to the other end of the Kan<sup>R</sup> gene so that the transcription from the CMV promotor proceeds in the clockwise orientation. A synthetic oligonucleotide segment containing translation initiation sequence and sequences encoding the human tissue plasminogen  
30 activator signal peptide (TPA) was used to link the CMV promotor and the sequences encoding the gp140 of the primary isolate HIV-1<sub>BX08</sub>. The gp140 sequence encodes a portion of the envelope protein between amino acid 33 and

666 which ends before the transmembrane domain of gp41 (see Figure 2). A translation termination codon was placed at the end of the gp140 coding sequence. Next to the gp140 coding region is a 0.2 kb fragment containing the bovine growth hormone (BGH) polyadenylation signal sequence that is PCR amplified from pRC/CMV (Invitrogen). A remnant 80 bp DNA segment from the SV40 polyadenylation signal remained between the BGH poly A sequence and the SacI site of pBluescript SK due to DNA manipulation and it serves no purpose in this plasmid.

The pCMV.gp140.BX08 construct was introduced into HB101 competent cells according to manufacturer's recommendations (GibcoBRL). Correct molecular clones were identified by restriction and sequencing analysis and their expression of gp140 was examined in transient transfections followed by Western blot analysis.

All DNAs used for immunizations were prepared using EndoFree Plasmid Kit (Qiagen). For intramuscular immunizations in mice, 100 µg of pCMV.gp140.BX08, in 100 µl PBS was injected into the tibialis anterior muscles at 4 weeks intervals. Gene gun immunizations were accomplished with the Helios Gene Gun System (Biorad). Cartridges were prepared according to manufacturer's recommendations. Specifically, each cartridge was made to contain 0.7 µg of the DNA and 0.5 mg gold. Immunizations were carried out by applying two cartridges to each animal onto the shaved abdominal area at 4 week intervals.

#### Example 2

This Example illustrates the synthesis of peptides.

Solid phase peptide synthesis of peptide CLP-501 and CLP-504 were conducted on an ABI 430A automated peptide synthesizer according to the manufacturer's standard protocols. The peptides were cleaved from solid support by treatment with liquid hydrogen fluoride in the presence of thiocresole, anisole, and methyl sulfide. The crude products were extracted with trifluoroacetic acid (TFA) and precipitated with diethyl ether.

The amino acid sequences of these peptides are shown in Table 1.

### Example 3

This Example illustrates *in vitro* cell culture protocols to re-stimulate and expand CTLs and assay for their effector functions.

Spleens of BALB/c mice injected with the plasmid, pCMV.gp140.BX08, prepared and formulated as described in Example 1, using the intramuscular route or gene gun delivery method, were removed 10 to 11 days post final booster injection. Spleenocytes at  $3.0 \times 10^7$  were co-cultured with  $1.3 \times 10^7$  autologous LPS blasts which had been pulsed with the test peptide for 5hr at 37°C and irradiated at 3000 rads in 10.0 ml of complete medium (RPMI 1640 supplemented with 10.0% 56°C heat-inactivated bovine serum, 120.0 units per ml of penicillin G sodium, 120.0 µg per ml of streptomycin sulphate and 0.35 mg per ml of L-glutamine) in a 25 cm<sup>2</sup> tissue culture flask. The cultures were kept at 37°C in a humidified CO<sub>2</sub> incubator for days, and the responders were then tested against peptide-pulsed P815 target cells in a standard *in vitro* 4 hr CTL assay as follows:

The responders were harvested from the 7-day cultures and washed once with RPMI 1640 medium without added bovine serum. The positive target was created by incubating 3 to 5 x 10<sup>6</sup> P815 cells with 100.0 µg of the specified peptide overnight in a 26°C water bath. The target cells were then labeled with <sup>51</sup>Cr at 250.0 uCi per 1 x 10<sup>6</sup> cells in the presence of 25.0 µg of the same test peptides for 60 to 75 minutes at 26°C. After washing twice with complete medium to remove excess <sup>51</sup>Cr, the targets were incubated at 2.5 x 10<sup>3</sup> with different numbers of the responders per well in a V-bottomed 96 well tissue culture plates for 4 hr in a 37°C CO<sub>2</sub> incubator. Half amount of the supernatant from each micro-assay culture was then removed and counted for radioactivity.

Results were expressed as % which was calculated using the equation:  
% lysis = (spontaneous lysis in cpm of experimental sample – spontaneous lysis in cpm of labeled target cells alone) divided by (total lysis in cpm of target cells alone – spontaneous lysis in cpm of target cells alone) x 100.

The results obtained employing intramuscular injection are shown in Figures 3A, 3B and 3C while those obtained employing the gene gun delivery are shown in Figures 4A, 4B and 4C.

Example 4

This Example illustrates the construction of alphavirus expression vectors pMP83, pMP84 and pMP88.

DNA vector pCMV.gp140.BX08, prepared as described in Example 1, was  
5 digested with restriction endonucleases NheI and BamHI to release the HIV-1  
gp140 sequence. The NheI/BamHI fragment was gel purified and ligated to a  
synthetic oligonucleotide linker made from the following annealed  
oligonucleotides: Oligo 1 - TPA-1  
5'- TCC GGA TCC ACC ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT  
10 GTG CTG CTG CTG TGT GGA GCA GTC TTC GTT TCG G -3' (SEQ ID No: 16)  
Oligo 2 - TPA-2  
5'- CTA GTC GAA ACG AAG ACT GCT CCA CAC AGC AGC AGC ACA CAG  
CAG AGC CCT CTC TTC ATT GCA TCC ATG GTG GAT CCG GA -3' (SEQ ID  
No: 17). This ligation restored the TPA signal sequence. The resulting fragment  
15 was restricted with BamHI and the resulting BamHI fragment was gel purified.  
The nucleotide sequence of the BamHI fragment is shown in Figure 5 (SEQ ID  
No: 15).

After the DNA sequence verification, the BamHI fragment was cut out of  
the pUC19 vector and ligated into BamHI restricted plasmids pMP42, pMP74 and  
20 pMP76 to make plasmids pMP88, pMP84 and pMP83 respectively.

Plasmid pMP42, containing the SFV replicon, is described in WO  
99/25858 (1038-864), assigned to the applicants and the disclosure of which is  
incorporated herein by reference and has been deposited with ATCC (203461).  
The construction of pMP42 is shown in Figures 2A and 2B of WO 99/25858.

25 Plasmid pMP76, containing the SFV replicon, is described in WO  
99/25859 (1038-862), assigned to the applicants and the disclosure of which is  
incorporated herein by reference and has been deposited with ATCC (203462).  
The construction of pMP76 is shown in Figures 8A to 8D of WO 99/25859.

Plasmid pMP74, containing the SFV replicon, is identical to pMP76  
30 except that it lacks the rabbit  $\beta$ -globin intron II insertion into the SFV replicon.  
This plasmid may be constructed by suitable modification to the scheme shown in  
Figures 8A to 8D of WO 99/25859.

### Example 5

This Example shows the results of immunizations using the alphavirus vectors.

5 The recombinant alphavirus vectors pMP88, pMP84 and pMP83, prepared as described in Example 4, were employed in comparative immunogenicity studies with plasmid pCMV.gp140.BX08, prepared as described in Example 1, in BALB/c mice following the procedure outlines in Example 1 for intramuscular immunization in mice using pCMV.gp140.BX08 and the CTL assay of Example 3, with unmodified pMP76 and pCMV being employed as negative controls. The  
10 results obtained are shown in Table II below.

Comparative analysis of the alphavirus constructs and the DNA construct of Example 1 showed similar results in the CTL assay. As expected, the negative control vectors that did not contain the gp140 sequences from HIV-1 BX08 showed no specific lysis in the CTL assay. All three alphavirus replicons, pMP83,  
15 pMP84 and pMP88, showed specific lysis as did the vector pCMV.gp140.BX08. The difference between the two types of vector was the amount of immunizing nucleic acid needed to elicit the same response. At 1 µg dose, the alphavirus vectors pMP83 and pMP88 showed comparable responses to pCMV.gp140.BX08 at a much higher dose of 100 µg.

20 These results were confirmed by an vector from a interferon-gamma (IFN-γ) assay, the results of which are shown in Table III. The assay is well known, as the measure of IFN-γ secreted from the spleenocytes indicated activation of the CTLs. Again, the alphavirus vectors showed comparable activation at an approximately 100 fold lower dose than the pCMV.gp140.BX08 vector. Overall  
25 these results indicate that immunization with nucleic acid vector expressing the gp140 sequence from the primary isolate BX08 generated MHC Class I restricted cytotoxic T-cells and that the alphavirus expression system used was approximately 100-fold more effective at the lower dose.

### SUMMARY OF DISCLOSURE

30 In summary of this disclosure, the present invention provides a novel plasmid expressing, *in vitro* and *in vivo*, the gp140 protein of the primary HIV-1

isolate BX08 and the generation of MHC class I-restricted cytotoxic T-cells in animals. Modifications are possible within the scope of this invention.



Table 1

MHC class I-restricted motifs of the extracellular envelope fragment, gp140, of HIV-1(BX08)

H-2 <sup>d</sup> -restricted *		HLA-A0201-restricted **	
Peptide ***	D <sup>d</sup> .	Peptide ***	K <sup>d</sup> .
CLP-501	ICPGRAFYTT (274-283) (SEQ ID No:3)	CLP-503	AYDTEVHNV (29-37) (SEQ ID No:4)
		CLP-504	FYSLKIVPI (141-149) (SEQ ID No:5)
		CLP-505	LYKYKVVVKI (443-451) (SEQ ID No:6)
		CLP-506	KYKVVVKIEPL (445-454) (SEQ ID No:7)
		CLP-507	RYLQDQRFL (545-553) (SEQ ID No:8)
		CLP-508	NYTEIYSL (597-605) (SEQ ID No:9)
			KLTPLCVTL (91-98) (SEQ ID No:10)
			TLFRVAIKL (305-313) (SEQ ID No:11)
			TLTVQARQL (403-411) (SEQ ID No:12)
			TLTVQARAL (496-504) (SEQ ID No:13)
			QLQARVLAL (535-543) (SEQ ID No:14)

\* or \*\* Anchors residues were typed in bold letters.

\*\*\* Peptides chosen for the study reported herein are bolded.

Table II  
A comparative immunogenicity study of recombinant alpha viruses and pCMV.gp140.BX08 expressing gp140 of HIV-1<sub>BX08</sub> in BALB/c mice

Immunization (3 injections, im)	Dose (µg)	% CLP-501 specific lysis at E:T ratio of 75:1
pMP76	100.0	0.6
	10.0	0.02
	1.0	0
pMP83	100.0	23.4
	10.0	38.5
	1.0	42.7
pMP84	100.0	37.2
	10.0	18.6
	1.0	12.4
pMP88	100.0 (not done, mice died)	
	10.0	39.6
	1.0	49.6
pCMV3	100.0	0
	10.0	0.06
	1.0	0
PCMV.gp140.BX08	100.0	59.7
	10.0	29.3
	1.0	0

Table III  
Frequencies of splenic CLP-501-specific effectors induced by recombinant vectors expressing HIV-1<sub>gX08</sub>  
gp140 determined by IFN- $\gamma$  ELISPOT

Immunization (3 injections, im)	Dose ( $\mu$ g)	Number of spots scored per $5 \times 10^5$ responders in duplicate cultures
pMP76	100.0	0, 0
	10.0	0, 0
	1.0	0, 0
pMP83	100.0	10, 14
	10.0	14, 19
	1.0	18, 13
pMP84	100.0	7, 12
	10.0	4, 7
	1.0	2, 6
pMP88	100.0 (not done, mice died)	
	10.0	20, 13
	1.0	24, 17
pCMV3	100.0	0
	10.0	0
	1.0	0
PCMV gp140.BX08	100.0	17, 13
	10.0	6, 9
	1.0	0

REFERENCES

1. B.J. Spalding. Biotechnology, vol. 10, pp 24-28, 1992
2. H.L. Robinson and C.A.T. Torres. Seminars in Immunology, vol. 9, pp 271-283, 1997.
3. Ian A. Wilson and Daved H. Fremont. Seminars in Immunology, vol. 5, pp 75-80, 1993.
4. Kristen Falk and Olaf Rotzschke. Seminars in Immunology. Vol. 5, pp 81-94, 1993.
5. Victor H. Engleford. Current Opinion in Immunology, Vol. 6, pp 13-23, 1994.
6. Tang et al, Nature, 1992, 356:152-154.
7. Furth et al, Analytical Biochemistry, 1992, 205:365-368.

CLAIMS

What we claim is:

1. A vector, comprising a gene encoding the extracellular fragment of gp140 of a primary HIV-1 isolate under the control of a promotor for expression of the gene product in a host organism.
2. The vector of claim 1, wherein said primary HIV-1 isolate is BX08.
3. The vector of claim 2, wherein the promoter is the cytomegalovirus promoter.
4. The vector of claim 1, which has the identifying characteristics of pCMV.gp140.BX08 (ATCC No. 203839), as shown in Figure 1.
5. The vector of claim 1, which has the identifying characteristic of pMP83, pMP84 or pMP88.
6. An immunogenic composition comprising a vector comprising a gene encoding the extracellular fragment of gp140 of a primary HIV-1 isolate under the control of a promoter for expression of the gene product in a host organism.
7. The immunogenic compositions of claim 6, wherein said primary HIV-1 isolate is BX08.
8. The immunogenic compositions of claim 6, wherein the promoter is the cytomegalovirus promoter.
9. The immunogenic composition of claim 6, wherein the vector is pCMV.gp140.BX08.
10. The immunogenic composition of claim 6 wherein the vector is pMP83, pMP84 or pMP88.
11. The immunogenic composition of claim 6 formulated for intramuscular immunization with a pharmaceutically-acceptable liquid carrier.
12. The immunogenic composition of claim 6 formulated for gene gun delivery with gold particles
13. A method of generating a cytotoxic T-cell response to HIV-1 in a host, which comprises administering to the host the immunogenic composition of claim 6.
14. The vector according to claim 1 when used as an immunogen for generating a cytotoxic T-cell response to HIV-1 in a host.

15. The use of a vector according to claim 1 in the manufacture of an immunogen for generating a cytotoxic T-cell response to HIV1 in a host.
16. A peptide having an amino acid sequence selected from the group consisting of SEQ ID Nos.: 3 to 14, as shown in Table 1.
17. The peptide of claim 16, having SEQ ID No.:3.
18. The peptide of claim 16, having SEQ ID No.:5.
19. A nucleic acid molecule having a nucleotide sequence selected from the group consisting of:
  - (a) a nucleotide sequence having SEQ ID No: 1 or the complementary sequence thereto;
  - (b) a nucleotide sequence encoding a gp140 protein having SEQ ID No: 2 or the complementary sequence thereto; and
  - (c) a nucleotide sequence having SEQ ID No: 15 or the complementary sequence thereto.

The pCMV.gp140.BX08 plasmid

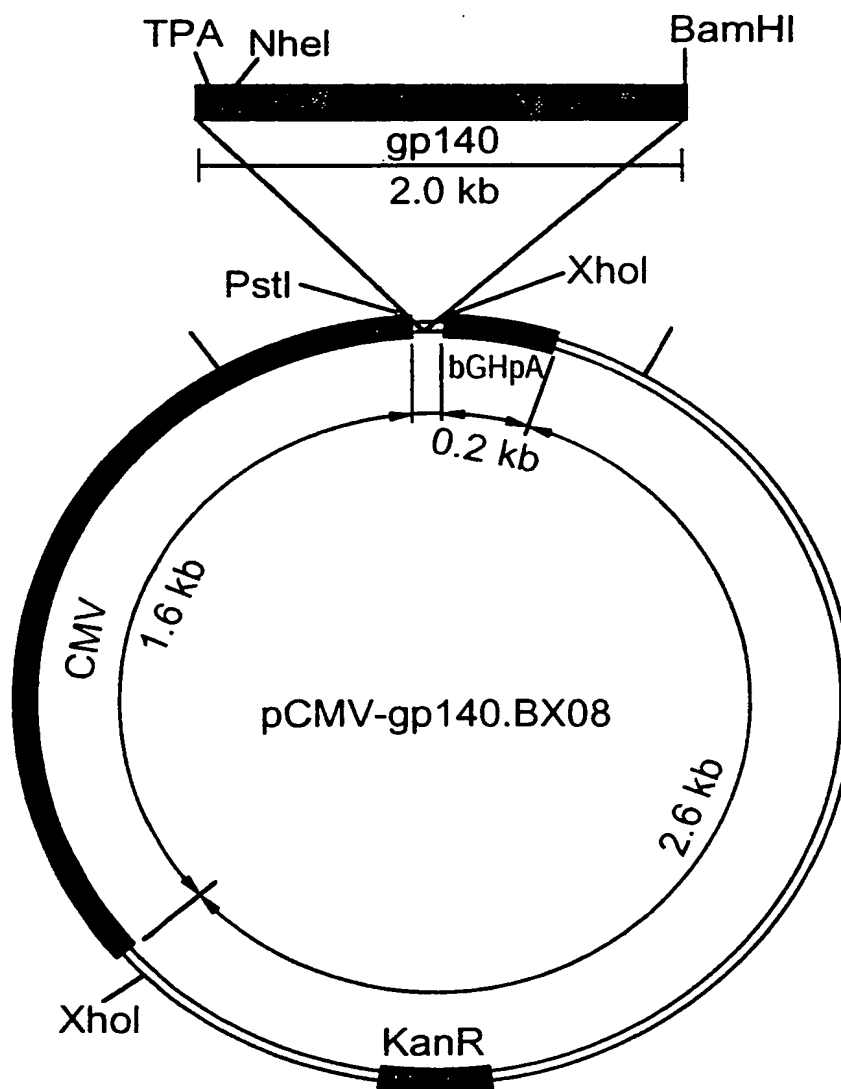


FIG.1

FIG.2A

```

+1 M D A M K R G L C C V L L L L C G
1  ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT GIG CTG CTG TGT GGA
   TAC CTA CGT TAC TTC TCT CCC GAG ACG ACA CAC GAC GAC GAC ACA CCT

+1 A V F V S A S L W V T V Y Y G V
49 GCA GTC TTC GTT TCG GCT AGC TTG TGG GTC ACA GTC TAT TAT GGG GTA
   CGT CAG AAG CAA AGC CGA TCG AAC ACC CAG TGT CAG ATA ATA CCC CAT

+1 P V W K E A T T T L F C A S D A
97 OCT GTG TGG AAA GAA GCA ACC ACC ACT CTA TTT TGT GCA TCA GAT GCT
   GGA CAC ACC TTT CTT CGT TGG TGG TGA GAT AAA ACA CGT AGT CTA CGA

+1 K A Y D T E V H N V W A T H A C
145 AAA GCA TAT GAT ACA GAA GTA CAT AAT GTT TGG GCC ACA CAT GCC TGT
   TTT CGT ATA CTA TGT CTT CAT GTA TTA CAA ACC CGG TGT GTA CGG ACA

+1 V P T D P N P Q E V V L G N V T
193 GTA CCC ACA GAC CCC AAC CCA CAA GAA GTA GTA TTG GCA AAT GTG ACA
   CAT GGG TGT CTG GGG TTG GGT GTT CTT CAT CAT AAC CCT TTA CAC TGT

+1 E N F N M G K N N M V E Q M H E
241 GAA AAT TTT AAC ATG GGG AAA AAT AAC ATG GTA GAA CAG ATG CAT GAA
   CTT TTA AAA TIG TAC CCC TTT TTA TIG TAC CAT CTT GTC TAC GTA CTT

+1 D I I S L W D Q Q S L K P C V K L
289 GAT ATA ATT AGT TTA TGG GAT CAA AGC CTA AAG CCA TGT GTA AAA TTA
   CTA TAT TAA TCA AAT ACC CTA GTT TCG GAT TTC GGT ACA CAT TTT AAT

```



FIG.2B

```

+1  T P L C V T L N C T K L K N S T
337 ACC CCA CTC TGT GTT ACT TTA AAT TGC ACT AAG TTG AAG AAT AGT ACT
    TGG GGT GAG ACA CAA TGA AAT TTA ACG TGA TTC AAC TTC TTA TCA TGA

+1  D T N N T R W G T Q E M K N C S
385 GAT ACC AAT AAT ACT ACA TGG GCA ACA CAA GAA ATG AAA AAC TGC TCT
    CTA TGG TTA TTA TGA TCT ACC OCT TGT GTT CTT TAC TTT TTG ACG AGA

+1  F N I S T S V R N K M K R E Y A
433 TTC AAC ATC AGC ACA AGT GTA ACA AAT AAG ATG AAG ACA GAA TAT GCA
    AAG TTG TAG TCG TGT TCA CAT TCT TTA TTC TAC TTC TCT CTT ATA CGT

+1  L F Y S L D I V P I D N D N T S
481 CTT TTT TAT AGT CTT GAT ATA GTA CCA ATA GAT AAT GAT AAT ACT AGC
    GAA AAA ATA TCA GAA CTA TAT CAT GGT TAT CTA TTA CTA TTA TGA TCG

+1  Y R L R S C N T S I I T Q A C P
529 TAT AGG TTA AGA AGT TGT AAT ACC TCA ATC ATT ACA CAG GCC TGT CCA
    ATA TCC AAT TCT TCA ACA TTA TGG AGT TAG TAA TGT GTC CCG ACA GGT

+1  K V S F E P I P I H F C A P A G
577 AAG GTA TCC TTT GAG CCA ATT CCC ATA CAT TTT TGT GCC CCG GCT GGT
    TTC CAT AGG AAA CTC GGT TAA GGG TAT GTA AAA ACA CCG GGC CCA CCA

+1  F A I L K C N N K T F N G T G P
625 TTT GCG ATT CTA AAG TGT AAC AAT AAA ACG TTC AAT GCA ACA GCA CCA
    AAA CCG TAA GAT TTC ACA TTG TTA TTT TGC AAG TTA CCT TGT CCT GGT

```

## FIG.2C

```

+1 C T N V S T V Q C T H G I R P V
673 TGT ACA AAT GTC AGC ACA GTA CAA TGT ACA CAT GGA ATT AGG CCA GTA
ACA TGT TTA CAG TCG TGT CAT GTT ACA TGT GTA CCT TAA TCC GGT CAT

+1 V S T Q L L L L N G S L A E E V
721 GTA TCA ACT CAA CTG CTG TTA AAT GGC AGC CTA GCA GAA GAG GTA
CAT AGT TGA GTT GAC GAC AAT TTA CCG TCG CAT CGT CTT CTC CAT

+1 V I R S E N F T N N A K T I I V
769 GTA ATT AGA TCT GAA AAT TTC ACA AAC AAT GCT AAA ACC ATA ATA GTA
CAT TAA TCT AGA CTT TTA AAG TGT TIG TTA CGA TTT TGG TAT TAT CAT

+1 Q L N E S V E I N C T R P N N N
817 CAG CTA AAT GAA TCT GTA GAA ATT AAT TGT ACA AGA CCC AAC AAC AAT
GTC GAT TTA CTT ACA CAT CTT TAA TTA ACA TGT TCT GGG TIG TIG TTA

+1 T R K S I H I G P G R A F Y T T
865 ACA AGA AAA AGT ATA CAT ATA GGA CCA GGG AGA GCA TTT TAT ACA ACA
TGT TCT TTT TCA TAT GTA TAT CCT GGT CCC TCT CGT AAA ATA TGT TGT

+1 G D I I G D I R Q A H C N I S R
913 GGA GAT ATA ATA GGA GAT ATA ACA CAA GCA CAT TGT AAC ATT AGT AGA
CCT CTA TAT TAT CCT CTA TAT TCT GTT CGT GTA ACA TIG TAA TCA TCT

+1 T N W T N T L K R V A E K L R E
961 ACA AAC TGG ACT AAC ACT TTA AAA AGG GTA GCT GAA AAA TTA ACA GAA
TGT TIG ACC TGA TIG TGA AAT TTT TCC CAT CGA CTT TTT AAT TCT CTT

```

## FIG.2D

```

+1 K F N N T T I V F N Q S S G G D
1009 AAA TTT AAT AAT ACA ATA GTC TTT AAT CAA TCC TCA GGA GGG GAC
    TTT AAA TTA TTA TGT TGT TAT CAG AAA TTA GTT AGG AGT CCT CCC CTG

+1 P E I V M H S F N C G G E F F Y
1057 CCA GAA ATT GTA ATG CAC AGT TTT AAT TGT GGA GGG GAA TTT TTC TAC
    GGT CTT TAA CAT TAC TAC GIG TCA AAA TTA ACA CCT CCC CTT AAA AAG ATG

+1 C N T T Q L F N S T W N E T N S
1105 TGT AAT ACA ACA CAA CTG TTT AAT AGT ACT TGG AAT GAA ACT AAC AGT
    ACA TTA TGT TGT GTT GAC AAA TTA TCA TCA ACC TTA CTT TCA TTG TCA

+1 E G N I T S G T I T L P C R I K
1153 GAA GGA AAT ATC ACC AGT GGA ACT ATA ACA CTC CCA TGC AGA ATA AAA
    CTT CCT TTA TAG TGG TCA CCT TGA TAT TGT GAG GGT ACG TCT TAT TTT

+1 Q I I N M W Q E V G K A M Y A P
1201 CAA ATT ATA AAC ATG TGG CAG GAA GTA GGA AAA GCA ATG TAT GCC CCT
    GIT TAA TAT TTG TAC ACC GTC CTT CAT CCT TTT OGT TAC ATA CCG GGA

+1 P I G G Q I K C L S N I T G L L
1249 CCC ATC GGA GGA CAA ATT AAA TGT TTG TCA AAC ATC ACA GGG CTG TTA
    GGG TAG CCT CCT GTT TAA TTT ACA AAC AGT TTG TAG TGT CCC GAC AAT

+1 L T R D G G S D N S S S G K E I
1297 TTA ACA ACA GAT GGT GGT AGT GAT AAC AGT AGT AGT GGG AAA GAG ATC
    AAT TGT TCT CTA CCA CCA TCA TTG TCA TCA CCC TTT CTC TAG

```

## FIG.2E

```

+1 F R P G G G D M R D N W R S E L
1345 TTC AGA CCT GGA GGG GGA GAT ATG AGG GAC AAT TGG AGA AGT GAA TTA
    AAG TCT GGA CCT CCC CCT CTA TAC TCC CTG TTA ACC TCT TCA CTT AAT

+1 Y K Y K V V K I E P L G I A P T
1393 TAT AAA TAT AAG GTA AAA ATT GAA CCA TTA GGA ATA GCA CCC ACC
    ATA TTT ATA TTC CAT TTT TAA CTT GGT AAT CCT TAT CGT GGG TGG

+1 K A K R R V V Q R E K R A V G I
1441 AAG GCA AAG AGA AGA GIG GIG CTG AGA GAA AAA AGA GCA GIG GGA ATA
    TTC CGT TTC TCT TCT CAC CAC GIC TCT CTT TTT TCT CGT CAC CCT TAT

+1 G A M F L G F L G A A G S T M G
1489 GGA GCC ATG TTC CTT GGG TTC TTG GGA GCA GCA AGC ACT ATG GGC
    CCT CCG TAC AAG GAA CCC AAG AAC CCT CGT CGT TCG TGA TAC CCG

+1 A A S L T L T V Q A R Q L L S G
1537 GCA GCG TCA ACG CTG ACG GTA CAG GCC AGA CAA TTA TTG TCT GGT
    CGT CGC AGT GAT TGC GAC TGC CAT GIC CGG TCT GTT AAT AAC AGA CCA

+1 I V Q Q Q N N L L R A I E A Q Q
1585 ATA GIG CAG CAG CAA AAC AAT TTG CTG AGG GCT ATT GAG GCG CAA CAG
    TAT CAC GIC GIC GGT TTG TTA AAC GAC TCC CGA TAA CTC CGC GTT GIC

+1 H L L L Q L T V W G I K Q L Q A R
1633 CAC CTG TTG CAA CTC ACA GTC TGG GGC ATC AAG CAG CTC CAG GCA AGA
    GIG GAC AAC GTT GAG TGT CAG ACC CCG TAG TTC GIC GAG GIC CGT TCT

```

FIG.2F

```

+1 V L A L E R Y L Q D Q R F L G M
1681 GTC CTG GCT CTG GAA ACA TAC CTA CAG GAT CAA CCG TTC CTA GCG ATG
CAG GAC CGA GAC CTT TCT ATG GAT GTC CTA GTT GGC AAG GAT CCC TAC

+1 W G C S G K L I C T T A V P W N
1729 TGG GGT TGC TCT CGA AAA CTC ATC TGC ACC ACT GCT GIG OCT TGG AAT
ACC CCA ACG AGA CCT TTT GAG TAG ACG TGG TGA CGA CAC GGA ACC TTA

+1 A S W S N K N L S Q I W D N M T
1777 GCT AGT TGG AGT AAT AAA AAT CTA AGT CAG ATT TGG GAT AAC ATG ACC
CGA TCA ACC TCA TTA TTT TTA GAT TCA GIC TAA ACC CTA TIG TAC TGG

+1 W M E W E R E I S N Y T E I I Y
1825 TGG ATG GAG TGG GAG ACA GAA ATA AGC AAT TAC ACA GAG ATA ATA TAT
ACC TAC CTC ACC CTC TCT TCT TAT TAT TGG TTA ATG TGT CTC TAT TAT ATA

+1 S L I E E S Q N Q Q E K N E L D
1873 AGC TTA ATT GAA GAA TCG CAG AAC CAA CAA GAA AAG AAT GAA CTA GAC
TGG AAT TAA CTT CTT AGC GTC TIG GTT GTT CTT TTC TTA CTT GAT CTG

+1 L L L Q L D K W A S L W N W F D I
1921 TTA TTA CAA TTG GAT AAG TGG GCA AGT TIG TGG AAT TGG TTT GAC ATA
AAT AAT GTT AAC CTA TTC ACC CGT TCA AAC ACC TTA ACC AAA CTG TAT

+1 T
1969 ACA
TGT

```

**Note:** shown is the translation of the sense strain with it's anti-sense strain below it that was cloned into the pQMV3 vector described in the text.

Effector responses elicited by intramuscular injection of the plasmid, pCMV.gp140.BX08, into BALB/c mice

<b>Immunization:</b> 100.0 ug pCMV.gp.BX08 + 100.0 ug pCMV	100.0 ug pCMV.gp140.BX08 100.0 ug pCMV	100.0 ug pCMV
<b>In vitro re-stimulation:</b> with autologous CLP-501 pulsed LPS blasts (■)  with autologous LPS Blasts (▲)	with autologous CLP-504 pulsed LPS blasts (●)	with autologous CLP-501 pulsed LPS blasts (▲)  with autologous CLP-504 pulsed LPS blasts (●)
<b>Target:</b> CLP-501-pulsed P815	CLP-504-pulsed P815	CLP-501-pulsed P815 for CLP-501-pulsed blast re-stimulation  CLP-504-pulsed P815 for CLP-504-pulsed blast re-stimulation

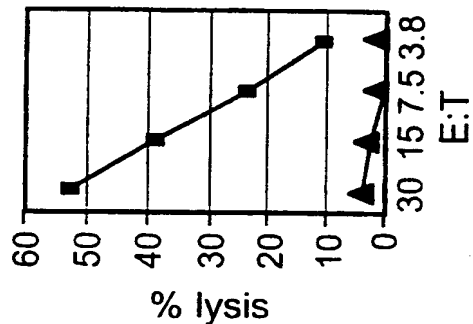


FIG.3A

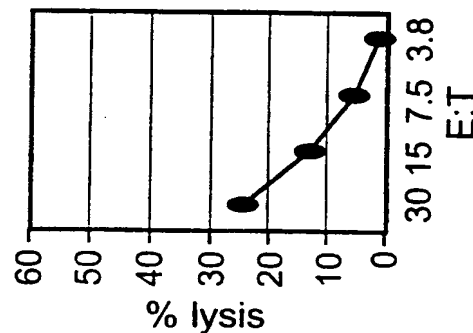


FIG.3B

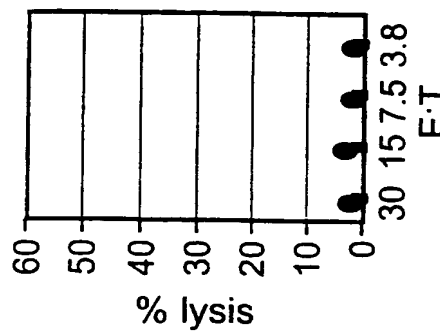


FIG.3C

Effector responses elicited by gene gun delivery of the plasmid, pCMV.gp140.BX08, into BALB/c mice

<b>Immunization:</b>	0.7 ug pCMV.gp140.BX08 + 0.7 ug pCMV	0.7 ug pCMV.gp140.BX08 + 0.7 ug pCMV	1.4 ug pCMV
<b>In vitro re-stimulation:</b>	with autologous CLP-501 pulsed LPS blasts (■)	with autologous CLP-504 pulsed LPS blasts (●)	with autologous CLP-501 pulsed LPS blasts (■)
	with autologous LPS Blasts (▲)		with autologous CLP-504 pulsed LPS blasts (●)
<b>Target:</b>	CLP-501-pulsed P815	CLP-504-pulsed P815	CLP-501-pulsed P815 for CLP-501-pulsed blast re-stimulation CLP-504-pulsed P815 for CLP-504-pulsed blast re-stimulation

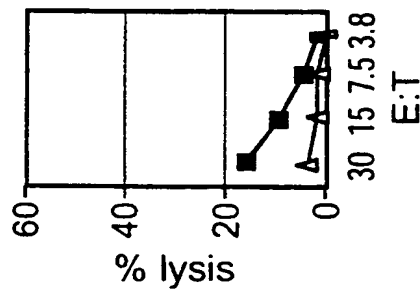


FIG. 4A

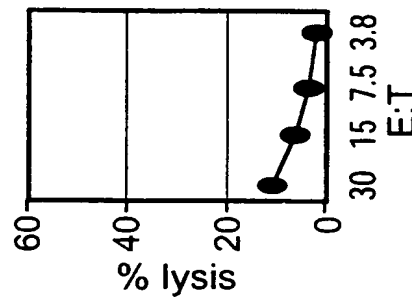


FIG. 4B

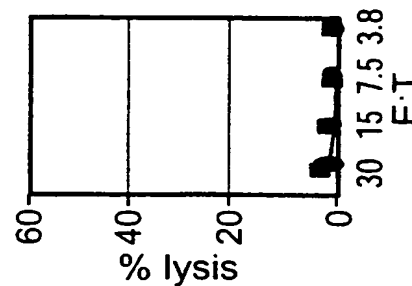


FIG. 4C

Sequence of Bam HI fragment containing HIV-1 gp 140 sequences for alphavirus expression vectors.

[illegible]

**FIG. 5**



# INTERNATIONAL SEARCH REPORT

Inter. Appl. Application No  
PCT/CA 00/00190

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/49 C07K14/16 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LU S ET AL: "Immunogenicity of DNA vaccines expressing human immunodeficiency virus type 1 envelope glycoprotein with and without deletions in the V1/2 and V3 regions." AIDS RESEARCH AND HUMAN RETROVIRUSES, JAN 20 1998, 14 (2) P151-5, XP000907375 UNITED STATES	1,3,6,8, 11,15
Y	abstract; figure 1	2,4,7,9
X	WO 93 18055 A (US HEALTH) 16 September 1993 (1993-09-16) page 7, line 16; claims 1,5,12,28	16,17
X	WO 97 31115 A (DAVIES MARY ELLEN ;PERRY HELEN C (US); SHIVER JOHN W (US); FREED D) 28 August 1997 (1997-08-28)	1,3,15
A	claims 1-10; figure 1; example 7	6,8,11
-/--		

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

29 May 2000

Date of mailing of the international search report

14/06/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3018

Authorized officer

Gurdjian, D

# INTERNATIONAL SEARCH REPORT

Internal Application No  
PCT/CA 00/00190

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YAHN ET AL: "STRUCTURAL VARIABILITY OF ENV AND GAG GENE PRODUCTS FROM A HIGHLY CYTOPATHIC STRAIN OF HIV-1" ARCHIVES OF VIROLOGY 1992, vol. 125, no. 1-4, 1992, pages 287-298, XP000907410 ISSN: 0304-8608	1,3,15
A	the whole document	6,8,11
Y	VERRIER FLORENCE C ET AL: "Antibodies to several conformation-dependent epitopes of gp120/gp41 inhibit CCR-5-dependent cell-to-cell fusion mediated by the native envelope glycoprotein of a primary macrophage-tropic HIV-1 isolate." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 1997, vol. 94, no. 17, 1997, pages 9326-9331, XP002138893 ISSN: 0027-8424 abstract; figure 1	2,4,7,9
A	WO 94 28929 A (GENENTECH INC ;BERMAN PHILLIP W (US); NAKAMURA GERALD R (US)) 22 December 1994 (1994-12-22) page 32 -page 35; claims 1-33	1-7,15, 19
A	O'BRIEN W A ET AL: "HIV-1 TROPISM FOR MONONUCLEAR PHAGOCYTES CAN BE DETERMINED BY REGIONS OF GP120 OUTSIDE THE CD4-BINDING DOMAIN" NATURE (LONDON) 1990, vol. 348, no. 6296, 1990, pages 69-73, XP002138883 ISSN: 0028-0836 the whole document	1-7,15, 19
A	-& EMBL DATABASE ; ACCESSION NUMBER U63632,30 August 1996 (1996-08-30), XP002138884 figure GP160	1-7,15, 19
	-/-	

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/CA 00/00190

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WESTERVELT P ET AL: "IDENTIFICATION OF A DETERMINANT WITHIN THE HUMAN IMMUNODEFICIENCY VIRUS 1 SURFACE ENVELOPE GLYCOPROTEIN CRITICAL FOR PRODUCTIVE INFECTION OF PRIMARY MONOCYTES" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 1991, vol. 88, no. 8, 1991, pages 3097-3101, XP002138885 ISSN: 0027-8424 the whole document	1-7,15, 19
A	-& EMBL DATABASE ; ACCESSION NUMBER M60472, 1 May 1991 (1991-05-01), XP002138886 the whole document	1-7,15, 19

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/CA 00 00190

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

Continuation of Box I.1

Although claims 13 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition. are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

# INTERNATIONAL SEARCH REPORT

information on patent family members

Inter. Appl. Application No

PCT/CA 00/00190

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9318055 A	16-09-1993	US 5976541 A	02-11-1999
		AU 668927 B	23-05-1996
		AU 3787893 A	05-10-1993
		CA 2131153 A	16-09-1993
		EP 0630385 A	28-12-1994
		US 5932218 A	03-08-1999
WO 9731115 A	28-08-1997	AU 2124697 A	10-09-1997
		BG 102784 A	31-05-1999
		BR 9707672 A	13-04-1999
		CN 1216064 A	05-05-1999
		CZ 9802667 A	17-03-1999
		EP 0904380 A	31-03-1999
		HR 970092 A	30-04-1998
		HU 9901112 A	28-07-1999
		NO 983876 A	21-10-1998
		PL 328730 A	15-02-1999
WO 9428929 A	22-12-1994	AU 700371 B	07-01-1999
		AU 7047894 A	03-01-1995
		EP 0708659 A	01-05-1996
		NZ 267838 A	19-12-1997
		US 6042836 A	28-03-2000
		US 5864027 A	26-01-1999

**THIS PAGE BLANK (USPTO)**